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Short review:

In situ method to estimate local effect of nasal preparations

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Introduction

Intranasal administration of drugs are mainly used to provide local effect in the nasal mucosa or underlying tissue. Systemic therapy, utilizing drug absorption from the nasal cavity is not widely practised, but some drugs, such as oxytocin, are now delivered via the nasal route. Nasal drops and sprays are used extensively for frequently occurring diseases such as the common cold and hay fever. These drugs are usually simple aqueous solutions containing substances with antiseptic, local analgesic and vasoconstrictor properties, when they are intended to act locally. Since the beginning of 1980's, the nasal route began to attract attention as a potential drug delivery site. Many drugs are rapidly absorbed from the nasal mucosa, allowing this route to be used to provide a rapid systemic response after intranasal administration. The extensive network of blood capillaries under the nasal membrane seems to facilitate effective systemic absorption of drugs, including highly molecular weight drugs such as insulin (6000 Dalton). This route of administration will probably have great potential for the future development of e.g. peptide preparations.

The mucociliary clearance is one of the most important defence mechanism of the human body against inhaled dust, allergens and microorganisms. Some pathophysiological conditions affects this mechanism, such as rhinitis, common cold, hay-fewer, sinusitis, asthma, nasal polyposis, Sjögren's and Kartagener's Syndrome. Furthermore, many environmental conditions, such as humidity, temperature, airborne toxins, as well as chemicals and many pharmaceutical excipients also affects this mechanism (*Hermens & Merkus* 1987, *Batts et al.* 1989, *Gizurarson et al.* 1990). To study the action of airborne toxins, chemicals, pharmaceuticals etc. on ciliary movement, a good method must be used, which gives information about the mucociliary clearance and of the ciliary movement. Theoretically, any treatment given topically in the nose has a potential to influence mucociliary clearance and therefore the defence mechanism of the human body. Some *in vitro* methods have been developed to measure the effect of chemical substances on the ciliary movement. Unfortunately, all the methods require living tissue, organs or an *in situ* method.

The frog palate method

This method was first described by *Sadé et al.* in 1970, where a solution, containing the testing substance, is dropped on the upper palate of a frog. This is followed by a visual measurement, where the time taken for graphite particles to travel a given distance is recorded. The influence of various substances on the mucociliary transport rate may be used to estimate the toxicity of the substance (*Gizurarson et al.* 1990). This model is similar as the "sugar test" in humans, as described later. The model is only able to show the affect on the mucociliary movement, but cannot give information how it effects the cilia or the mucus layer. For example in a study by *Van de Donk et al.* (1980) chlorhexidine gluconate irreversibly halted the beating of cilia, where measured

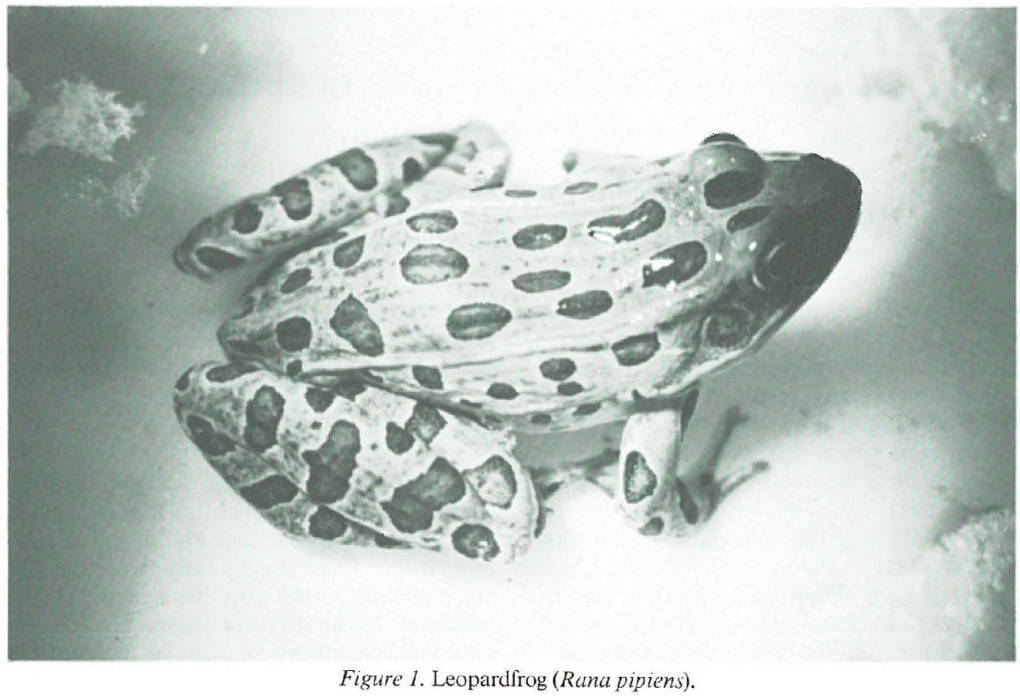

Figure 1. Leopardfrog (*Rana pipiens*).

on the frog palate method (*Batts & Marriott* 1988), the mucociliary clearance was still apparant. Indicating that in this case it is likely that the mucus is protecting the underlying epithelium from such toxic compounds. Therefore, substances that are able to affect the mucociliary clearance in this model, may be studied further in other models to find out if the effect is due to changes in the mucus layer or direct effect on the cilia.

Briefly, the procedure is as follows: *Rana pipiens, R. escilenta, R. temporaria, R. ridibunda* or *Bufo viridis* may be used (Fig. 1). The frog or the toad is made unconscious by a blow on the head. Immediately after, it is beheaded and the lower jaw is cutted off. The head (palate preparation) is placed in a transparent chamber, maintained at 22°C ± 1°C with a relative humidity of 100 %. The palate surface is observed through a stereomicroscope with calibrated eyepiece (Fig. 2). Control solution for all compounds is phosphate-buffered saline (PBS) and the test solutions are prepared by dissolving the required amount of compound in the appropriate control solution. Before each experiment 200 µl of the PBS solution is applied to the palate and after approximately 2 minutes, drained off. Control values (n = 4) for the transport rate is then measured by recording the time taken for graphite particles to be tansported a given distance along the midline of the palate (0.5 mm). The particles are placed on the anterior part of the hard palate, just behind the vomerine teeth. Cilia are thereafter transporting the particles towards the posterior part near the oesophagus. Palate preparations having low transport rate (< 0.1 mm/s) before the experiment should be discorded. Between each exposure the palate is rinsed with PBS. Preparations having no ciliary movement 30 minutes after exposure of the test compound, followed by 3 washing, should be considered as toxic to the mucoliary movement. To

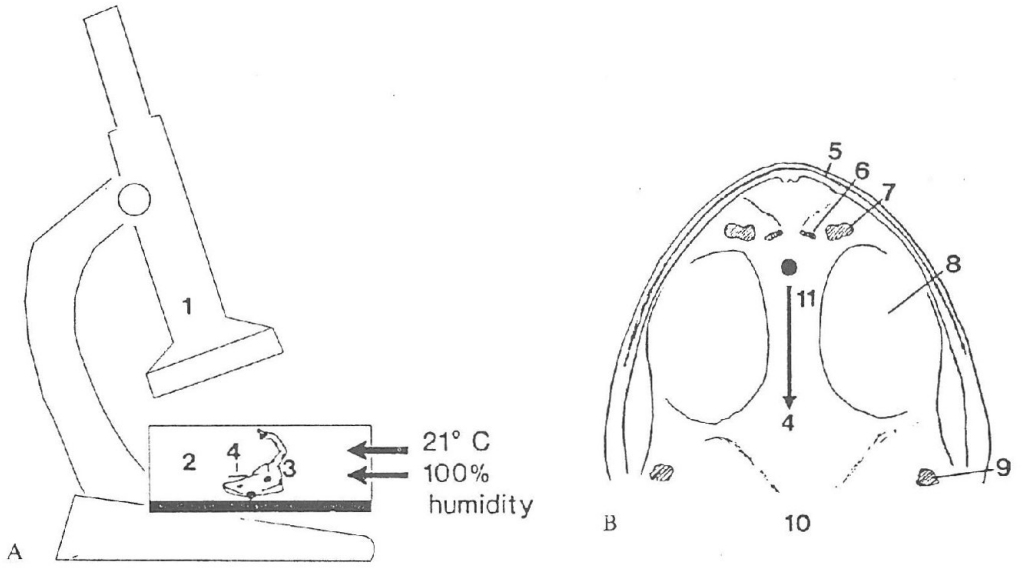

Figure 2. A: The experimental set-up for the frog palate model; B: The roof of the frogs mouth; C: Photograph of the roof of Leopardfrogs (*Rana pipiens*) mouth. 1. Stereo microscope; 2. Chamber, maintained at 21°C with relative humidity of 100 %; 3. Pithed frog with upper palate exposed; 4. Region of transport for the graphite particles; 5. Maxillary teeth; 6. Vomerine teeth; 7. Nasal passage (choana); 8. Protrusion of eyeballs; 9. Eustacian tube leading to the middle ear;10. Entrance to oesophagus; 11. Hard palate.

evaluate the influence on cilia activity from each test substance, each palate preparation must be used as its own control.
In this model, the cilia are protected by an intact layer of mucus. Which results in the effect on the complete mucociliary transport system. On the other hand, the results seems to correlate with those obtained with the ciliary beat frequency model, described by *Hermens et al.* (1990).
Comparing results from human nasal irritation studies with the results from the frog palate model, this model seems to be able to identify the least irritating excipients and formulations and may provide a basis for the selection of a nontoxic formulations.

Alternative methods

Ferret

Anaesthetized ferret is placed in the supine position on a heating pad. A longitudinal incision of about 2 cm is made in the neck to expose the underlying intact trachea. To measure the mucociliary transport a 5 µl droplets, containing microaggregated radiolabelled albumin, are deposed onto the dorsal surface of the trachea distalt to two detectors. The detectors are collimated to monitor two discrete areas, each 6.5 mm long, separated by a longitudinal distance of 19 mm. Each detector is connected to a count rate meter which measured the length of time in minutes for the radioactivity to pass between the two detectors (*Marin et al.* 1989). This method is similar as the frog palate method and may give the same result. The method require more expensive animal and more advanced instrumentation may be required. On the other hand, the method shows the transport in a living animals, where all functions are provided with blood and other important factors, which are not the case in the frog palate model.

Pigeons

Unanaesthetized pigeon is placed in the supine position and the trachea surgically exposed under local anaesthetic. 1 cm below the larynx, a 2 cm part of the trachea is opened and fixed, whereafter the pigeon is placed into an observation box with 38° C and 100 % humidity. Particles of wet cork (80–120 µm in diameter) are placed on the caudal side of the tracheal mucosa and the measurement starts when the cork transport velocity become constant. The time taken for the cork particles to move 10 mm is measured (*Miyata et al.* 1987, *Kai et al.* 1989). This method is similar as the ferret method. For measurements like these, a full anaesthesia may be required, except when the anaesthesia affects the results.

Human subjects

Human subjects are used as the final check for the effect of drugs on the mucoliary clearance. This method is commonly used to study disease stages where the ciliary beat frequency is affected, as well as after toxic exposure of environmental toxins, gases, hazards or after long time smoking.
To study the effect of substances, healthy volunteers are used. Particles of saccharine with blue dye are placed on the anteriomedial portion of the inferiour turbinate, at a distance of 4 cm from the nasal tip. The time between application of saccharine and sweet taste in the mouth define the transport time, which is confirmed by arrival of blue dye in the pharynx. Subjects are told not to sniff during the procedure. The length of the cavity from the nasal tip to pharyngeal wall is determined with a cotton swab and the velocity (mm/min) is calculated (*Anderson et al.* 1974, *Holmberg & Pipkorn* 1986).

In vitro methods

In vitro methods are methods where a living tissue is isolated from a sacrificed animal in order to measure the ciliary beat frequency. After exposure of drugs on the tissue surface, containing ciliated cells, the beats may be recorded and compared with a control. The results indicate the effect of the substance on the mobility, energy or the structure of the cilia. These methods may re-

quire a large amounts of animals for each experimental series. For all methods, described below, the system contain both cilia and mucus, except for the chicken embryo method where the mucus has not yet been produced. These systems may, therefore, give a realistic indication for the effect on the mucociliary transport in living species and subjects (*Van de Donk et al.* 1982).

Chicken embryo. This method is commonly used for the measurement of ciliary beat frequency. Chicken embryo tracheas are isolated and sliced into rings, whereafter a light beam is transmitted through the moving cilia and after magnification by a microscope the flickering light is projected on a photocell and the frequency is estimated electronically and displayed. The mucus has not been produced in these chicken embryo speciments and therefore this system may detect the direct effect of substances on the cilia.

Guinea pigs. Anaesthetized guinea pigs are sacrificed and the nasal cavities are exposed in order to access the septum. A sample of the mucosal epithelium is removed and a 5 mm window is placed on an adhesive tape on a microscope slide. The beating frequency of the cilia is then recorded by micro-photo-oscillograph where all changes in the ciliary beat frequency may be recorded (*Levrier et al.* 1989).

Rabbit. Rabbit maxillary sinus mucosa specimens are removed after killing the animal. Each specimens are about 5×5 mm in size. The tissue sample is placed on a humid surface where the mucociliary wave frequency may be recorded by the variation in the surface light reflections brought about by the movements of cilia. All changes in the ciliary beat frequency may be recorded (*Reimer* 1987).

Human specimens. Surgical procedures in the upper respiratory tract may give tissue samples that may be used similarly as described above. Adenoid tissue from children is probably the easiest one to collect after adenoidectomy. Slices about 1 mm thick are inspected for the presence of motile cilia. Whereafter a light beam is transmitted through the moving cilia and after magnification by a microscope the flickering light is projected on a photocell and the frequency is estimated electronically and displayed (*Van de Donk et al.* 1982, *Hermens et al.* 1987, *Hermens et al.* 1990). A drawback for this method may be the drug used for local anaesthesia (if used) or the anaesthesia, since most drugs affect the cilia. Anaesthesia such as nitrous oxide/oxygen may have minimal effect on the preparations. A disadvantage for this preparations may be that the adenoid samples used, are usually infected or have caused some kind of purpose for removal.

Cell culture. Recently, some cell cultures have been produced from rabbit tracheas (*Sanderson & Dirksen* 1989) and human nasal polyps (*Jorissen et al* 1989). Unfortunately, it is diffucult to design those systems with the same density of each cell type, as in normal tissue and the lack of support from nearstanding cells and the underlying tissue may influence the results. After culturing ciliated cells on collagen gels, the culture cells are observed with phase-contrast optics using a water immersion objective (×40). The ciliary beat frequency is measured using a photoelectronic technique.

Conclusion

All experiments should be designed, depending on the purpose and the aim of the study, the characteristics of the substances and how the results should be used. Simple methods, giving the required answer are often the methods not used, because they do not require complicated instrumentation and little challenge for the researcher. From a scientific point of view, the models giving the largest amount of different data are the most interesting. Giving the researcher different data to work with, whereas in clinical phases, simple toxic evaluations are enough. Before going to clinical phase, the frog palate model may be preferred, because it shows the effect

Table 1. The effect of various substances in the ciliary response.

Substance	Ciliary response
Drugs	
Acetylcholine	Increase the activity
Atropin (oral administration)	Drying, cessation of ciliary activity
Cocaine	Paralyzes cilia
Diphenhydramine	Paralyzes cilia
Ephedrine	No effect
Halothane	Decrease the activity
Insulin	No effect
Nitrous oxide	No effect
Pilocarpine	Increase the activity
Excipients	
Benzalkonium chloride	Decrease the activity
Chlorbutol	Decrease the activity
L-α-lysophosphatidylcholine	Paralyzes cilia
Oils	Interferes with ciliary motion
Polyoxyethylene-9-lauryl ether	Paralyzes cilia
Sodium deoxycholate	Paralyzes cilia
Sodium glycocholate	No effect
Thiomersal	No effect
Tween 20	Increase the activity
Infection	
Haemophilus influenza	No effect

of the material on the complete system as well as giving indication for the irritation level in humans. Substances that affects this system may be studied further in one of the previously discussed models, where the effect on the ciliary beat frequency or the effect on the mucus layer may be measured. Table 1 shows the effect of various substances on the ciliary response.

Summary

The potential local toxicity to the respiratory mucosa of drugs, excipients and formulations should be tested in an appropriate animal model. Today, many models are available for such studies, giving different information. Since the mucociliary clearance is one of the most important defence mechanism of the human body against inhaled dust, allergens and microorganisms, this mechanism may not be affected or destroyed. Environmental conditions, such as humidity, temperature, airborne toxins, as well as chemicals and many pharmaceutical excipients affects this mechanism. This review gives a short insight into this field, focusing mainly on the frog palate model, where the effect of drugs on the mucociliary transport rate may be studied.

Resumé

Lokal toksisitet fra lægemidler, hjælpestoffer og formuleringer, på den respiratoriske mukosa, skal undersøges i en egnet dyremodel. Idag findes mange modeller til disse undersøgelser, der giver forskellige informationer. Da den mukociliary clearance er en af kroppens vigtigste forsvarsmekanismer mod støv, allergener og mikroorganismer, må denne mekanisme ikke blive påvirket eller ødelagt. Forskellige omstændigheder, som fugtighed, temperatur, toksiner i luften, eller kemikalier og mange farmaceutiske hjælpestoffer påvirker denne mekanisme. Denne oversigtsartikel giver lidt indsigt ind i dette forskningsområde, med fokus hovedsagelig på "frøgane-modellen", hvor påvirkningen af lægemidler på den mukociliary transporthastighed kan undersøges.

Ágrip

Staðbundin áhrif á slimhinnur öndunarfæranna, af völdum lyfja, hjálparefna og lyfjaforma, skulu athuguð í þar til gerðum dýramódelum. Idag finnast mörg módel til þess að frankvæma þessar rannsóknir og gefa þær einnig mismunandi upplýsingar. Þar sem hreinsunarhæfni bifháranna er einn af mikilvægustu varnarkerfum líkamans gegn ryki, ofnæmisvaldandi egnum og örverum, má þetta kerfi ekki verða fyrir varanlegum áhrifum eða eyðileggingum. Mismunandi aðstæður, svo sem raki, hitastig, eiturefni í lofti, eða þá efni og mörg lyfjafræðileg hjálparefni, hafa áhrif á þetta

kerfi. Þessi yfirlitsgrein veitir stutta innsýn ínn í þetta rannsóknarsvið, með aðaláherslu á "froskagóms mælitæknina", þar sem hægt er að mæla ahrif lyfja á hreinsunarhæfni slimhimnunnar.

Yhteenveto / K. Pelkonen

Limakalvojen värekarvakuljetus on ihmisessä yksi tärkeimmistä suojamekanismeista sisäänhengitettyä pölyä, allergeeneja ja mikrobeja vastaan. Tämän vuoksi siihen ei saisa aiheuttaa häiriöitä. Kuljatukseen vaikuttavat monet ympäristötekijät, kuten ilmankosteus, lämpötila, ilmassa olevat myrkylliset aineet, kemikaalit ja farmakologiset vaikutusaineet. Tällaisten aineiden vaikutuksia hengityselimistän limakalvoon tulisi tutkia sopivassa eläinmallissa. Katsaus paneutuu lyhyesti aihepiiriin, ja erityisesti sammakonkitalakimalliin, jossa voidaan selvittää lääkeaineiden vaikutuksia limakalvon värekarvakuljetukseen.

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